

Phytochemical and Neuropharmacological Characterization of *Newbouldia laevis* Reveals GABAergic and Glycinergic Contributions to Its Antiseizure Activity

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Abstract

Newbouldia laevis is widely employed in African traditional medicine for the management of epilepsy and other convulsive disorders. Despite its extensive ethnomedicinal use, the neuropharmacological mechanisms underlying its anticonvulsant activity remain poorly defined. This study investigated the antiseizure potential of *N. laevis* leaf extract and chromatographic fractions and explored their possible involvement in GABAergic and glycinergic neurotransmission. The ethanol leaf extract of *N. laevis* was fractionated by column chromatography to obtain three fractions designated N-green, N-pink, and N-yellow. Phytochemical constituents were characterized using gas chromatography–mass spectrometry (GC–MS). Antiseizure activity was evaluated in mice using sodium barbital-induced hypnosis, picrotoxin-induced seizures, and strychnine-induced seizure models. Diazepam served as the reference drug. Seizure latency, seizure duration, sleep parameters, and 24-hour mortality were assessed. The crude extract and N-green fraction significantly potentiated sodium barbital-induced hypnosis, reducing sleep latency and prolonging sleep duration in a dose-dependent manner. In the picrotoxin model, both treatments significantly delayed seizure onset and reduced seizure duration, with the N-green fraction at 600 mg/kg producing effects comparable to diazepam. Furthermore, the crude extract and N-green fraction completely prevented picrotoxin-induced mortality at doses ≥ 300 mg/kg, whereas diazepam achieved only partial protection. In contrast, neither the extract, fractions, nor diazepam altered seizure parameters or prevented mortality in strychnine-treated mice. GC–MS analysis revealed the presence of several bioactive phytochemicals that may contribute to the observed neuropharmacological effects. *Newbouldia laevis* exhibits pronounced anticonvulsant and sedative activities, mediated predominantly through GABAA-AA-ergic rather than glycinergic mechanisms. These findings provide pharmacological support for its traditional use in epilepsy management and identify the bioactive fraction as a promising source of lead compounds for the development of novel GABA-targeted antiseizure therapies.

Keywords: *Newbouldia laevis*; Anticonvulsant, Epilepsy, GABA Receptor, Picrotoxin, Phytochemicals

1. Introduction

Epilepsy is one of the most common neurological disorders worldwide, affecting an estimated 50 million people across all age groups and socioeconomic settings [1-3]. Approximately 80% of individuals living with epilepsy reside in low- and

middle-income countries, where the burden of the disease is particularly high. Lifetime prevalence rates range from about 5.8 per 1000 population in developed countries to as high as 15.4 per 1000 in rural communities of developing nations [1, 4-6]. Characterized by recurrent and unprovoked seizures, epilepsy imposes substantial physical, psychological, social,

and economic challenges on affected individuals, their families, and healthcare systems [1].

Although considerable advances have been made in the development of antiseizure medications (ASMs), treatment outcomes remain suboptimal for many patients. It is estimated that 30–40% of individuals with epilepsy continue to experience seizures despite receiving appropriate pharmacological treatment, a condition referred to as drug-resistant epilepsy [7, 8]. In addition, many currently available ASMs are associated with undesirable adverse effects, including cognitive dysfunction, behavioural disturbances, and systemic toxicity, which may compromise treatment adherence and overall quality of life [9, 10]. These limitations underscore the need for the discovery of safer and more effective therapeutic alternatives.

Natural products have long served as an important source of drug leads and pharmacologically active compounds. Indeed, nearly half of all modern pharmaceuticals are either directly derived from or inspired by natural products [11–16]. Within this context, medicinal plants used in traditional healthcare systems have attracted increasing scientific attention as valuable resources for drug discovery and systems pharmacology research [11,16]. Ethnopharmacological investigations have already contributed significantly to epilepsy therapeutics, as exemplified by cannabidiol from *Cannabis sativa*, which has gained FDA approval for the treatment of certain forms of treatment-resistant epilepsy [17, 18].

Newbouldia laevis (P. Beauv.) Seem. (Bignoniaceae), commonly known as the African border tree or *Akoko* in many parts of West Africa, is one such medicinal plant with a longstanding history of use in the management of convulsive disorders [19, 20]. Indigenous to tropical Africa, the plant is widely utilized in traditional medicine, with its leaves, stem bark, and roots employed in the treatment of epilepsy, convulsions, and other neurological conditions [19, 20]. Experimental studies have provided preliminary evidence supporting these traditional claims. The leaf and root extracts of *Newbouldia laevis* were reported to significantly delay the onset of drug-induced seizures in mice, also demonstrated protective effects against electrically induced convulsions in rats [21–23]. Together, these findings suggest that *N. laevis* may possess clinically relevant anticonvulsant properties.

Phytochemical studies of *N. laevis* have revealed the presence of diverse classes of bioactive compounds, including flavonoids, alkaloids, saponins, and terpenoids [22, 23]. Notable constituents such as newbouldine, lapachol, and several flavonoid glycosides have also been isolated and characterized [23–25]. Despite these advances, detailed characterization of the plant's secondary metabolites using gas chromatography–mass spectrometry (GC–MS), together with a systematic investigation of their potential mechanisms of

anticonvulsant action, remains limited. This knowledge gap restricts our understanding of the bioactive principles responsible for the plant's pharmacological effects and hinders efforts to identify novel lead compounds for antiseizure drug development.

At the neurobiological level, epilepsy is widely understood as a disorder of neuronal hyperexcitability resulting from an imbalance between excitatory and inhibitory neurotransmission within the central nervous system [26–28]. Of particular importance are the inhibitory neurotransmitters γ -aminobutyric acid (GABA) and glycine, whose actions help regulate neuronal excitability and maintain network stability. Dysfunction of the GABAergic system has been strongly implicated in the pathogenesis of epilepsy, making enhancement of GABA-mediated inhibition a major therapeutic strategy employed by several clinically used ASMs, including benzodiazepines, barbiturates, and valproate [29, 30]. Similarly, although the glycinergic system has received comparatively less attention in epilepsy research, accumulating evidence suggests that it contributes significantly to seizure regulation, particularly through inhibitory control mechanisms in the brainstem and spinal cord [31].

Despite the growing body of evidence supporting the anticonvulsant potential of *N. laevis*, the neurochemical mechanisms underlying its effects remain poorly understood. In particular, whether its bioactive constituents exert their actions through modulation of GABAergic and/or glycinergic neurotransmission has not been adequately investigated. Elucidating these mechanisms is essential not only for validating the plant's traditional use but also for identifying novel therapeutic targets and bioactive molecules that may contribute to future antiseizure drug development.

Accordingly, the present study adopted an integrative approach combining phytochemical and pharmacological investigations to evaluate the anticonvulsant potential of *N. laevis*. The study involved GC–MS profiling of the crude leaf extract and its chromatographic fractions (N pink, N yellow, and N green), alongside assessment of their effects in rodent models of sodium barbital-, picrotoxin-, and strychnine-induced seizures. Survival analyses were also performed to further evaluate protective efficacy. Specifically, the objectives were to: characterize the phytochemical constituents of the crude extract and fractions using GC–MS analysis, determine their effects on sleep latency, sleep duration, seizure onset, seizure duration, and mortality; and investigate the relative contributions of GABAergic and glycinergic mechanisms to their anticonvulsant activity.

This approach is consistent with contemporary trends in natural product research, which emphasize mechanism-based pharmacological screening coupled with advanced analytical techniques for the identification of bioactive compounds

responsible for observed therapeutic effects. By integrating phytochemical characterization with mechanistic pharmacological evaluation, this study seeks to provide scientific validation for the traditional use of *N. laevis* in seizure management and to identify potential chemical scaffolds that may contribute to the development of safer and more effective antiseizure medications targeting GABAergic and glycinergic pathways. Such findings may ultimately help address the persistent unmet clinical need associated with drug-resistant epilepsy.

2. Materials and Methodology

2.1 Plant Collection and Identification

Fresh leaves of *Newbouldia laevis* were collected from the Botanical Garden of Bowen University Teaching Hospital, Ogbomoso, Nigeria. The plant material was identified and authenticated by taxonomists in the Department of Biology, Ladoke Akintola University of Technology (LAUTECH), Ogbomoso. A voucher specimen (LHO 389) was deposited for future reference.

2.2 Extraction and Fractionation

The collected leaves were thoroughly cleaned and air-dried at room temperature for three weeks. The dried leaves were then ground into a fine powder using a mechanical grinder. Extraction was carried out using a Soxhlet apparatus with 96% ethanol as the extraction solvent. Following extraction, the solvent was removed under reduced pressure using a rotary evaporator, and the resulting crude extract was stored at 4 °C until further analysis.

To facilitate fractionation, thin-layer chromatography (TLC) was first conducted to identify a suitable solvent system. A mixture of *n*-hexane and ethyl acetate (9:1, v/v) produced the best separation profile and was subsequently adopted for column chromatography. Separation of the crude extract on a silica gel column yielded three major fractions with differing polarities, designated as N-pink, N-yellow, and N-green. Based on chromatographic behaviour, N-pink represented the most polar fraction, N-yellow exhibited intermediate polarity, while N-green was the least polar. Both the crude extract and the isolated fractions were subsequently subjected to phytochemical and pharmacological evaluation.

2.3 Gas Chromatography–Mass Spectrometry (GC–MS) Analysis

The phytochemical composition of the crude extract and chromatographic fractions was investigated using gas chromatography–mass spectrometry (GC–MS). Analyses were performed on an Agilent 8860 Gas Chromatograph coupled to a 5977B Mass Selective Detector and fitted with an Elite-5MS capillary column (30 m × 0.25 mm internal diameter, 0.25 µm film thickness).

The injector temperature was maintained at 300 °C, while high-purity helium (99.999%) was used as the carrier gas at a constant flow rate of 1 mL/min. One microlitre of each sample was injected in split mode (10:1). The oven temperature programme was initiated at 110 °C and held for 1 minute, then increased at a rate of 15 °C/min to 310 °C and maintained for an additional 2 minutes. Mass spectral data were acquired at an ionization energy of 70 eV within a scanning range of 45–450 *m/z*. Compound identification was achieved by comparing the obtained mass spectra with those contained in the National Institute of Standards and Technology (NIST) spectral database.

2.4 Experimental Animals

Healthy adult male and female Wistar mice weighing between 20 and 25 g were obtained from the Animal House of the Department of Physiology, Bowen University, Nigeria. The animals were housed under standard laboratory conditions, maintained at a temperature of 25 ± 2 °C with a 12-hour light/dark cycle, and provided with commercial feed (Top Feeds®, Nigeria) and clean water *ad libitum*. Prior to the commencement of experiments, the animals were allowed to acclimatize to the laboratory environment for one week.

All experimental procedures were reviewed and approved by the Faculty of Basic Clinical Sciences Research Ethics Committee, Ladoke Akintola University of Technology, Ogbomoso. The study was conducted in accordance with the provisions of the European Union Directive 2010/63/EU for the care and use of laboratory animals.

2.4.1 Sodium Barbital-Induced Hypnosis Test

Mice were randomly allocated into experimental groups consisting of five animals per group. Prior to treatment, the animals were fasted for 12 hours while retaining free access to water. The crude extract and individual fractions of *N. laevis* were administered orally at doses of 150, 300, and 600 mg/kg. Thirty minutes after treatment, sodium barbital (45 mg/kg, intraperitoneally) was administered to induce hypnosis. The onset of sleep was assessed by measuring the time taken for the animals to lose the righting reflex (sleep latency), while sleep duration was recorded as the interval between the loss and recovery of the righting reflex [42].

2.4.2 Picrotoxin-Induced Convulsion

The anticonvulsant activity of the extract and fractions against GABAergic inhibition was evaluated using the picrotoxin-induced seizure model. Animals received oral administration of the crude extract or fractions at doses of 150, 300, or 600 mg/kg, 30 minutes before picrotoxin challenge (10 mg/kg) [33]. Diazepam (2 mg/kg) served as the positive control. Following picrotoxin administration, animals were observed for 30 minutes, during which seizure latency and seizure duration were recorded. Mortality was monitored for up to 24 hours after seizure induction.

2.4.3 Strychnine-Induced Convulsion

To investigate the possible involvement of glycinergic neurotransmission, seizures were induced using strychnine (2 mg/kg, i.p.). Mice were pretreated orally with the crude extract or fractions at doses of 150, 300, or 600 mg/kg, 30 minutes before administration of strychnine. Diazepam (2 mg/kg) was used as the reference anticonvulsant drug, while distilled water (10 mL/kg) served as the negative control. Animals were observed for seizure onset, seizure duration, and mortality using the same procedures described for the picrotoxin-induced seizure model [34].

2.5 Statistical Analysis

Data obtained from the experiments were expressed as mean $\pm$ standard error of the mean (SEM). Statistical analyses were performed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc multiple comparison test where appropriate. Differences were considered statistically significant at a probability level of $p < 0.05$.

3. Results

3.1 Pharmacological and Phytochemical Characterization of *Newbouldia laevis*

The pharmacological and phytochemical evaluations of *Newbouldia laevis* (*N. laevis*) crude extract and its chromatographic fractions, N-Pink, N-Yellow, and N-Green demonstrated distinct biological activities across the experimental models employed. The extract and fractions exhibited varying degrees of sedative-hypnotic and anticonvulsant effects, with responses generally showing dose dependence. In addition, GC-MS analysis revealed a diverse phytochemical composition, suggesting that multiple bioactive constituents may contribute to the observed pharmacological actions.

3.2 Effects of *Newbouldia laevis* Extract and Fractions on Sodium Barbital-Induced Sleep in Mice

In the vehicle-treated control group, animals exhibited a mean sleep latency of 14.63 ± 2.55 minutes and a mean sleep duration of 49.46 ± 7.41 minutes. As expected, diazepam (2 mg/kg) produced a marked sedative-hypnotic effect, significantly shortening sleep latency to 4.41 ± 0.82 minutes while extending sleep duration to 107.42 ± 14.60 minutes.

Administration of the crude extract (N-Crude) and the N-Green fraction at doses of 300 and 600 mg/kg significantly ($p < 0.05$) enhanced sodium barbital-induced hypnosis. These treatments reduced the time required for the onset of sleep, with latency values ranging from 4.85 to 7.50 minutes, and increased sleep duration to between 72.00 and 98.07 minutes. The magnitude of these effects approached those observed with diazepam treatment, indicating substantial central nervous system depressant activity. The N-Pink fraction produced a more modest response. A significant reduction in sleep latency was

observed only at the highest dose tested (600 mg/kg), where latency decreased to 6.43 ± 0.65 minutes. However, its effects were generally less pronounced than those of the crude extract and N-Green fraction. In contrast, the N-Yellow fraction did not significantly affect either sleep latency or sleep duration at any of the doses evaluated (Tables 1–4).

Overall, these findings suggest that the sedative-hypnotic activity of *N. laevis* is concentrated primarily within the crude extract and the N-Green fraction. The stronger activity observed in these preparations may be attributable to relatively non-polar phytochemicals capable of potentiating sodium barbital-induced central nervous system depression, whereas the N-Yellow fraction appears to contain fewer or lower concentrations of compounds involved in this effect.

Table 1: Effect of 'N-Crude' on sodium barbital hypnosis in mice

Treatment	Dose (Mg/kg)	Sleep latency(min)	Duration of sleep (min)
Control	10	14.63 ± 2.55	49.46 ± 7.41
Diazepam	2	$4.41 \pm 0.82^*$	$107.42 \pm 14.60^*$
N-Crude	150	$11.20 \pm 1.61^*$	68.44 ± 6.30
N-Crude	300	$6.82 \pm 0.94^*$	$72.00 \pm 10.05^*$
N-Crude	600	$4.85 \pm 0.21^{* \#}$	$96.72 \pm 12.11^{* \#}$

Values represent mean $\pm$ SEM (n = 5); * $p < 0.05$ compared with the control. $^{\#}p > 0.05$ compared with diazepam. Control = 1% dimethyl sulfoxide (1% DMSO), which is the vehicle; N-Crude = *Newbouldia laevis* crude extract

Table 2: Effect of N-Green on sodium barbital hypnosis in mice

Treatment	Dose(mg/kg)	Onset of sleep (min)	Duration of sleep (min)
Control	10	14.63 ± 2.55	49.46 ± 7.41
Diazepam	2	$4.41 \pm 0.82^*$	$107.42 \pm 14.60^*$
N-Green	150	$8.74 \pm 2.40^{* \#}$	$60.40 \pm 8.04^{* \#}$
N-Green	300	$7.50 \pm 1.21^{* \#}$	$84.33 \pm 5.53^*$
N-Green	600	$5.32 \pm 1.46^*$	$98.07 \pm 10.21^*$

Table 3: Effect of 'N-Pink' on sodium barbital hypnosis in mice

Treatment	Dose (Mg/kg)	Onset of sleep (min)	Duration of sleep (min)
Control	10	14.63 ± 2.55	49.46 ± 7.41
Diazepam	2	$4.41 \pm 0.82^*$	$107.42 \pm 14.60^*$
'N-Pink'	150	10.54 ± 1.04	54.26 ± 4.11
'N-Pink'	300	9.22 ± 1.12	67.76 ± 6.12
'N-Pink'	600	$6.43 \pm 0.65^*$	$82.56 \pm 5.33^*$

Values represent mean $\pm$ SEM (n = 5); * $p < 0.05$ compared with the control. $^{\#}p < 0.05$ compared with diazepam. Control = 1% dimethyl sulfoxide (1% DMSO), which is the vehicle; N-Pink = *Newbouldia laevis* pink fraction

3.3 Effects of *Newbouldia laevis* Extract and Fractions on Picrotoxin-Induced Seizures in Mice

The anticonvulsant potential of *N. laevis* was further evaluated using the picrotoxin-induced seizure model, which is widely employed to assess compounds that modulate GABAergic neurotransmission. Picrotoxin acts as a non-competitive antagonist of the GABAA_{AA} receptor chloride channel, thereby reducing inhibitory neurotransmission and

precipitating seizures. Agents capable of enhancing GABAergic activity are therefore expected to delay seizure onset and reduce seizure severity.

In the control group, picrotoxin administration produced seizures with a mean onset latency of 62.44 ± 8.01 seconds and a mean duration of 56.73 ± 6.42 seconds. Diazepam (2 mg/kg), used as the reference anticonvulsant drug, significantly ($p < 0.05$) delayed seizure onset to 96.56 ± 10.61 seconds and reduced seizure duration to 24.33 ± 7.05 seconds. Treatment with the crude extract (N-Crude) and the N-Green fraction produced marked anticonvulsant effects, particularly at doses of 300 and 600 mg/kg. These treatments significantly ($p < 0.05$) prolonged seizure latency, with values ranging from 84.65 to 98.24 seconds, while simultaneously reducing seizure duration to between 18.40 ± 6.40 and 31.00 seconds. The magnitude of protection observed was comparable to, and in some cases approached, that produced by diazepam.

The N-Pink fraction demonstrated moderate anticonvulsant activity. A significant protective effect was observed only at the highest dose tested (600 mg/kg), where seizure latency increased to 80.31 ± 8.25 seconds and seizure duration decreased to 31.90 ± 7.33 seconds. In contrast, the N-Yellow fraction did not produce significant changes in either seizure onset or seizure duration at any of the doses evaluated (Tables 5–8). Overall, the results indicate that the anticonvulsant activity of *N. laevis* is concentrated primarily within the crude extract and the N-Green fraction. The ability of these preparations to antagonize picrotoxin-induced seizures strongly suggests the involvement of GABAergic mechanisms, possibly through enhancement of GABAA_A-mediated inhibitory neurotransmission. These findings are consistent with previous reports demonstrating anticonvulsant effects of *N. laevis* in related chemoconvulsant models [35].

Table 4: Effect of N-Yellow on sodium barbital hypnosis in mice

Treatment	Dose(mg/kg)	Onset of sleep (min)	Duration of sleep (min)
Control	10	14.63 ± 2.55	49.46 ± 7.41
Diazepam	2	$4.41 \pm 0.82^*$	$107.42 \pm 14.60^*$
N-Yellow	150	13.34 ± 2.10	46.24 ± 3.32
N-Yellow	300	12.52 ± 1.50	42.66 ± 3.14
N-Yellow	600	16.14 ± 1.42	56.01 ± 5.20

Values represent mean $\pm$ SEM (n = 5); * $p < 0.05$ compared with the control. Control = 1% dimethyl sulfoxide (1% DMSO), which is the vehicle; N-Yellow = *Newbouldia laevis* yellow fraction.

3.4 Effects of *Newbouldia laevis* Extract and Fractions on Strychnine-Induced Seizures in Mice

The involvement of glycinergic neurotransmission in the anticonvulsant activity of *N. laevis* was investigated using the strychnine-induced seizure model. Strychnine produces convulsions by selectively antagonizing glycine receptors,

thereby disrupting inhibitory neurotransmission and promoting neuronal hyperexcitability.

Unlike the findings observed in the picrotoxin-induced seizure model, neither the crude extract nor any of the chromatographic fractions produced significant changes in seizure latency or seizure duration following strychnine administration. Similarly, diazepam, the reference drug, did not significantly modify seizure onset or duration in this model (Tables 9–12).

The lack of protective effects against strychnine-induced convulsions suggests that the anticonvulsant activity of *N. laevis* is unlikely to be mediated through direct modulation of glycine receptors or glycinergic neurotransmission. Rather, the findings support the notion that the plant's anticonvulsant properties are more closely associated with GABAergic mechanisms, as demonstrated in the picrotoxin model.

Together, these results indicate that while *N. laevis* possesses appreciable anticonvulsant activity, its effects appear to be selective and pathway-specific. The inability of the extract and fractions to protect against strychnine-induced seizures is consistent with previous reports suggesting that the pharmacological actions of *N. laevis* are largely confined to GABAergic and possibly opioidergic systems, with little or no direct involvement of glycinergic pathways under the conditions of this study.

Table 5: Effect of 'N-Crude' on picrotoxin-induced convulsion

Treatment	Dose(mg/kg)	Seizure latency (sec)	Seizure duration (sec)
Control	10	62.44 ± 8.01	56.73 ± 6.42
Diazepam	2	$96.56 \pm 10.61^*$	$24.33 \pm 7.05^*$
N-Crude	150	76.34 ± 6.42	45.81 ± 7.20
N-Crude	300	$84.65 \pm 10.11^{* \#}$	$30.33 \pm 4.55^{* \#}$
N-Crude	600	$92.71 \pm 6.10^{* \#}$	$18.40 \pm 6.40^{* \#}$

Values represent mean $\pm$ SEM (n = 5); * $p < 0.05$ compared with the control. $^{\#}p > 0.05$ compared with diazepam. Control = 1% dimethyl sulfoxide (1% DMSO), which is the vehicle; N-Crude = *Newbouldia laevis* crude extract

Table 6: Effect of N-Green on picrotoxin-induced convulsion

Treatment	Dose (mg/kg)	Seizure latency (sec)	Seizure duration (sec)
Control	10	62.44 ± 8.01	56.73 ± 6.42
Diazepam	2	$96.56 \pm 10.61^*$	$24.33 \pm 7.05^*$
N-Green	150	72.14 ± 8.30	48.50 ± 6.30
N-Green	300	$87.60 \pm 8.41^*$	$36.92 \pm 4.43^*$
N-Green	600	$98.24 \pm 6.55^*$	$26.35 \pm 2.73^*$

Values represent mean $\pm$ SEM (n = 5); * $p < 0.05$ compared with the control. Control = 1% dimethyl sulfoxide (1% DMSO), which is the vehicle; N-Green = *Newbouldia laevis* green fraction.

3.5 Effects of *Newbouldia laevis* Extract and Fractions on Seizure-Associated Mortality

The protective effects of *N. laevis* against seizure-related mortality were assessed following picrotoxin- and strychnine-induced convulsions. Marked differences were observed among

the treatment groups, further highlighting the selective anticonvulsant activity of the plant extract and its fraction

Table 7: Effect of N-Pink on picrotoxin-induced convulsion

Treatment	Dose (mg/kg)	Seizure latency (sec)	Seizure duration (sec)
Control	10	62.44 ± 8.01	56.73 ± 6.42
Diazepam	2	96.56 ± 10.61*	24.33 ± 7.05*
N-Pink	150	66.06 ± 5.70	54.22 ± 6.24
N-Pink	300	70.93 ± 8.07	48.00 ± 5.84
N-Pink	600	80.31 ± 8.25*	31.90 ± 7.33*

Values represent mean ± SEM (n = 5); *p<0.05 compared with the control. Control = 1% dimethyl sulfoxide (1% DMSO), which is the vehicle; N-Pink = *Newbouldia laevis* pink fraction

Table 8: Effect of N-Yellow on picrotoxin-induced convulsion

Treatment	Dose (mg/kg)	Seizure latency (sec)	Seizure duration (sec)
Control	10	62.44 ± 8.01	56.73 ± 6.42
Diazepam	2	96.56 ± 10.61*	24.33 ± 7.05*
N-Yellow	150	55.20 ± 3.51	61.58 ± 7.40
N-Yellow	300	58.20 ± 5.52	50.86 ± 6.23
N-Yellow	600	67.85 ± 9.43	44.64 ± 6.06

Values represent mean ± SEM (n = 5); *p<0.05 compared with the control. Control = 1% dimethyl sulfoxide (1% DMSO), which is the vehicle; N-Yellow = *Newbouldia laevis* yellow fraction

Table 9: Effect of 'N-Crude' on Strychnine-induced convulsion

Treatment	Dose (mg/kg)	Seizure latency (sec)	Seizure duration (sec)
Control	10	43.31 ± 4.70	12.54 ± 2.42
Diazepam	2	62.20 ± 6.22	14.40 ± 2.13
N-Crude	150	46.42 ± 5.65	14.06 ± 2.22
N-Crude	300	39.74 ± 3.35	18.93 ± 3.54
N-Crude	600	40.82 ± 4.10	16.11 ± 2.45

Values represent mean ± SEM (n = 5); Control = 1% dimethyl sulfoxide (1% DMSO), which is the vehicle; N-Crude = *Newbouldia laevis* crude extract

Table 10: Effect of 'N-Green' on strychnine-induced convulsion

Treatment	Dose (mg/kg)	Seizure latency (sec)	Seizure duration (sec)
Control	10	43.31 ± 4.70	12.54 ± 2.42
Diazepam	2	62.20 ± 6.22	14.40 ± 2.13
N-Green	150	36.80 ± 6.12	17.41 ± 3.22
N-Green	300	42.77 ± 8.40	16.25 ± 2.13
N-Green	600	48.63 ± 6.84	14.86 ± 2.55

Values represent mean ± SEM (n = 5); Control = 1% dimethyl sulfoxide (1% DMSO), which is the vehicle; 'N-Green' = *Newbouldia laevis* green fraction

In the picrotoxin-induced seizure model, all animals in the vehicle-treated control group succumbed within 24 hours, resulting in a mortality rate of 100%. Treatment with diazepam provided partial protection, with a survival rate of 60%. Notably, the crude extract (N-Crude) and the N-Green fraction demonstrated substantial protective effects. At doses of 300 mg/kg and above, both treatments completely prevented mortality, resulting in 100% survival of the animals. The N-Pink fraction also exhibited a degree of protection, reducing mortality and achieving an 80% survival rate at doses of 300 and 600 mg/kg. In contrast, the N-Yellow fraction failed to

confer any meaningful protection, with mortality rates comparable to those observed in the control group (**Table 13**). The strong survival benefits observed with the crude extract and N-Green fraction complement their ability to delay seizure onset and reduce seizure duration in the picrotoxin model, providing further evidence of significant anticonvulsant activity mediated through GABAergic mechanisms.

In contrast, no protective effects were observed in the strychnine-induced seizure model. Regardless of treatment, all animals succumbed following strychnine administration, resulting in a mortality rate of 100% across all experimental groups. This finding further supports the conclusion that *N. laevis* lacks appreciable activity against glycine receptor-mediated convulsions and associated lethality.

Overall, the mortality data reinforce the pharmacological findings from the seizure models, indicating that the anticonvulsant and neuroprotective effects of *N. laevis* are selective for GABAergic pathways, with little or no efficacy against seizures arising from disruption of glycinergic neurotransmission.

Table 11: Effect of 'N-Pink' on Strychnine-induced convulsion

Treatment	Dose (mg/kg. bw)	Seizure latency (sec)	Seizure duration (sec)
Control	10	43.31 ± 4.70	12.54 ± 2.42
Diazepam	2	52.20 ± 6.22	14.40 ± 2.13
N-Pink	150	33.45 ± 5.52	10.40 ± 2.50
N-Pink	300	28.20 ± 3.00	9.81 ± 1.74
N-Pink	600	36.63 ± 4.30	12.55 ± 2.11

Values represent mean ± SEM (n = 5); Control = 1% dimethyl sulfoxide (1% DMSO), which is the vehicle; N-Pink = *Newbouldia laevis* pink fraction

Table 12: Effect of 'N-Yellow' on strychnine-induced convulsion

Treatment	Dose (mg/kg bw)	Seizure latency (sec)	Seizure duration (sec)
Control	10	43.31 ± 4.70	12.54 ± 2.42
Diazepam	2	52.20 ± 6.22	14.40 ± 2.13
N-Yellow	150	28.15 ± 3.04	8.66 ± 1.42
N-Yellow	300	38.12 ± 5.23	16.10 ± 2.32
N-Yellow	600	46.72 ± 7.02	10.54 ± 2.63

Values represent mean ± SEM (n = 5); Control = 1% dimethyl sulfoxide (1% DMSO), which is the vehicle; N-Yellow = *Newbouldia laevis* yellow fraction

3.6 Phytochemical Profile of *Newbouldia laevis* Crude Extract and Chromatographic Fractions Using GC-MS

Gas chromatography–mass spectrometry (GC–MS) analysis of the crude extract and chromatographic fractions of *N. laevis* (Figure 1 A-D) revealed a chemically diverse profile comprising more than twenty identifiable compounds. The detected constituents belonged to several phytochemical classes, including terpenoids, fatty acid derivatives, esters, hydrocarbons, siloxanes, and other bioactive secondary metabolites.

Among the major compounds identified were benzoic acid methyl ester, which constituted approximately 25.88% of the total chromatographic area, and neophytadiene, a prominent constituent detected in both the crude extract (20.70%) and the N-Green fraction (34.21%). Other notable compounds included phytol, phytol acetate, bicyclohexane derivatives, and a range

of siloxane- and ester-containing molecules. The relative abundance of these compounds varied across the fractions, reflecting differences in their chemical composition and polarity. Of particular interest was the N-Green fraction, which exhibited the highest concentration of neophytadiene and appreciable levels of phytol-related compounds. Both neophytadiene and phytol have previously been reported to possess neuroactive properties, including anxiolytic, sedative, antioxidant, and anticonvulsant activities. Their enrichment within the N-Green fraction may therefore provide a plausible explanation for the pronounced sedative-hypnotic and anticonvulsant effects observed in this fraction during the sodium barbital- and picrotoxin-induced seizure studies.

The GC–MS findings suggest that the pharmacological activities of *N. laevis* are likely mediated by the combined actions of multiple phytoconstituents rather than a single active compound. Furthermore, the concentration of potentially neuroactive terpenoid compounds within the N-Green fraction appears to correlate with its superior biological activity, supporting the possibility that these constituents contribute significantly to the GABAergic-mediated anticonvulsant effects observed in the present study. Further isolation and mechanistic investigations will be necessary to identify the specific compounds responsible for these effects and to clarify their individual contributions to the overall pharmacological profile of *N. laevis*.

Table 13: Effect of ‘*Newbouldia laevis* Extract and Fractions on Seizure-Associated Mortality

Treatment	Dose	Picrotoxin (%)	Strychnine (%)
Control	10	100	100
Diazepam	2	40	100
N-Crude	150	20	100
N-Crude	300	0	100
N-Crude	600	0	100
N-Green	150	20	100
N-Green	300	0	100
N-Green	600	0	100
N-Pink	150	100	100
N-Pink	300	80	100
N-Pink	600	80	100
N-Yellow	150	100	100
N-Yellow	300	100	100
N-Yellow	600	100	100

Control = 1% dimethyl sulfoxide (1% DMSO), which is the vehicle; N-Crude = *Newbouldia laevis* crude extract, N-Green = *Newbouldia laevis* green fraction N-Yellow = *Newbouldia laevis* yellow fraction, N-Pink = *Newbouldia laevis* pink fraction

Discussion

The findings of the present study provide compelling evidence that *Newbouldia laevis* leaf extract and its chromatographic fractions possess significant anticonvulsant and sedative-hypnotic properties. These effects were largely dose-dependent and were most pronounced in the crude extract (N-Crude) and

the N-Green fraction. The observed pharmacological activities, particularly in the sodium barbital-induced hypnosis and picrotoxin-induced seizure models, strongly suggest that the anticonvulsant actions of *N. laevis* are mediated primarily through enhancement of GABAergic neurotransmission aligning with reports of previous studies [19, 21, 35, 36].

In the sodium barbital-induced hypnosis test, both the crude extract and N-Green fraction significantly reduced sleep latency and prolonged sleep duration, producing effects that closely approached those of diazepam corroborating the report of Amos *et al* [37]. Since sodium barbital exerts its hypnotic action through facilitation of GABA-mediated inhibitory neurotransmission, substances that potentiate GABAergic activity are expected to enhance its sedative effects [38, 39]. The ability of *N. laevis* preparations to augment sodium barbital-induced sleep therefore suggests an interaction with inhibitory neurotransmitter systems, particularly the GABAA_{AA} receptor complex.

This observation was further supported by the results obtained in the picrotoxin-induced seizure model. Picrotoxin acts as a non-competitive antagonist of the GABAA_{AA} receptor chloride channel and induces seizures by reducing inhibitory neurotransmission. Compounds capable of enhancing GABAergic signaling typically delay seizure onset and attenuate seizure severity in this model [19, 40]. In the present study, N-Crude and N-Green significantly prolonged seizure latency, reduced seizure duration, and markedly improved survival rates, with effects comparable to those produced by diazepam. These findings strongly indicate that the anticonvulsant activity of *N. laevis* is linked to facilitation of GABAA_{AA}-mediated inhibition.

The phytochemical profile obtained through GC–MS analysis provides a plausible mechanistic basis for these pharmacological effects. Among the major constituents identified were neophytadiene, phytol, phytol acetate, and benzoic acid methyl ester. Notably, neophytadiene and phytol were particularly abundant in the N-Green fraction, which consistently exhibited the greatest anticonvulsant and sedative activities. Previous studies have reported that both compounds possess neuroactive properties, including anxiolytic, sedative, antioxidant, and anticonvulsant effects [41, 42]. Their reported ability to influence GABAergic neurotransmission may therefore contribute substantially to the biological activities observed in the present study. Likewise, benzoic acid methyl ester, which was identified as a major constituent of the crude extract, has been associated with sedative and muscle-relaxant properties, further supporting the observed pharmacological responses.

An important finding of this study was the complete lack of protection afforded by the extract and its fractions against strychnine-induced seizures. Strychnine produces convulsions

by selectively antagonizing glycine receptors, thereby disrupting glycinergic inhibitory neurotransmission. Neither the crude extract nor any of the fractions significantly altered seizure latency, seizure duration, or mortality in this model. This selective inactivity suggests that the anticonvulsant effects of *N. laevis* are unlikely to involve direct modulation of glycine receptors. Rather, the data indicate a degree of pharmacological specificity for GABAergic pathways.

These findings are consistent with previous reports on the anticonvulsant mechanisms of *N. laevis*. Kolawole et al [35] demonstrated that the anticonvulsant effects of the plant could be reversed by flumazenil, a benzodiazepine receptor antagonist, whereas interventions targeting glycinergic pathways failed to produce similar effects. Such observations support the notion that *N. laevis* exerts its actions primarily through GABAA_{AA} receptor-associated mechanisms and possibly through interactions with opioid systems rather than glycinergic neurotransmission. Similarly, Usman et al[23] reported dose-dependent protection against pentylenetetrazole-induced seizures following administration of ethanolic extracts of *N. laevis*, further reinforcing the role of the plant in modulating seizure models associated with impaired GABAergic function.

The mortality data obtained in this study provide additional support for this interpretation. Complete protection against picrotoxin-induced mortality was achieved with N-Crude and N-Green at doses of 300 mg/kg and above, whereas mortality remained unchanged in the strychnine model regardless of treatment. The concordance between seizure suppression and survival outcomes highlights the functional significance of the GABAergic-mediated anticonvulsant effects and underscores the therapeutic potential of the plant in seizure conditions characterized by deficient inhibitory neurotransmission.

Together, the results of this study validate the traditional use of *Newbouldia laevis* in the management of convulsive disorders and provide important insights into its possible mechanisms of action. The superior activity of the N-Green fraction suggests that relatively non-polar phytochemicals, particularly neophytadiene and phytol-related compounds, may be key contributors to the observed pharmacological effects. However, the possibility of synergistic interactions among multiple phytoconstituents cannot be excluded.

Future studies should focus on the isolation and characterization of the major bioactive compounds identified in this work, followed by detailed receptor-binding and molecular pharmacology investigations. The use of pharmacological antagonists such as flumazenil and naloxone would further clarify the relative contributions of benzodiazepine-sensitive GABAA_{AA} receptor pathways and opioid mechanisms. Such studies may facilitate the identification of novel lead

compounds for the development of safer and more effective antiseizure therapies.

Overall, the present findings establish *N. laevis* as a promising source of bioactive compounds with significant GABAergic anticonvulsant activity and provide a strong pharmacological and phytochemical foundation for future drug-discovery efforts aimed at addressing epilepsy and related neurological disorders.

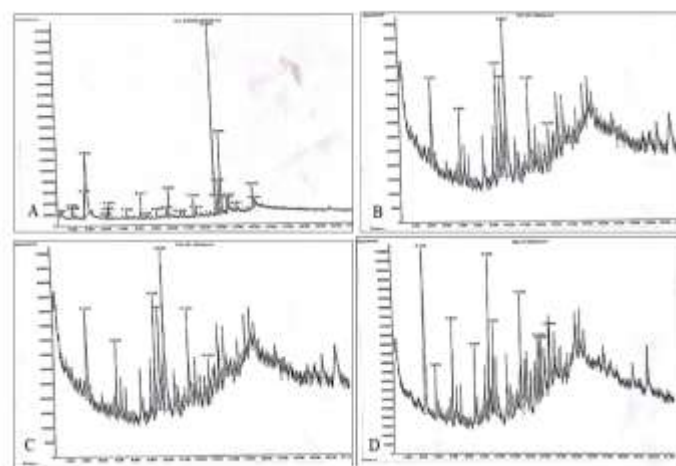


Figure 1 (A-D): Spectra of *Newbouldia laevis* (a) Crude extract, (b) Green, c) Pink and d) Yellow fraction

Conclusions

Newbouldia laevis leaf extract, particularly the N-Green fraction, exhibited significant dose-dependent anticonvulsant and sedative effects in rodent models involving GABAA_{AA}-mediated neurotransmission, with efficacy comparable to diazepam. The absence of activity against strychnine-induced seizures indicates limited involvement of glycinergic pathways. GC-MS analysis identified neophytadiene, phytol, and benzoic acid methyl ester as major constituents that may underlie these effects. These findings support the traditional use of *N. laevis* in seizure management and highlight its potential as a source of novel GABAergic anticonvulsant agents.

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None Declared

Conflict of Interest

Authors declare no conflict of interest

Availability of data and materials

Data generated during and analysed during the course of this study are available from the corresponding author on request.

Declaration of ethical approval

All procedures were followed as outlined in the guide for the Care and Use of Laboratory Animals published by the National Institute of Health.

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